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Kytococcus sedentarius and *Micrococcus luteus*: highly prevalent in indoor air and potentially deadly to the immunocompromised – *should standards be set?*

Folayan, A.[1], Mohandas, K.[2], Ambu, S.[3*], Kumarasamy, V.[4], Lee, N.[5] and Mak, J.W.[6]
[1,3,4,6]The International Medical University, No.126, Jalan 19/155B, Bukit Jalil, 57000 Kuala Lumpur, Malaysia
[2]MAHSA University, Jalan Elmu, Off Jalan Universiti, 59100 Kuala Lumpur, Malaysia
[5]Vinayaka Missions International University College, Block 2, 14, Jalan Utara, Pjs 52, 46200 Petaling Jaya, Selangor
[*]Corresponding author e-mail: stephen_ambu@imu.edu.my
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Abstract. The multifarious types of infections contracted from indoor environments show that buildings can serve as a reservoir for infectious bacteria. This study is an investigation into the type and concentrations of bacteria in the indoor and outdoor environments of an electronic factory, an office and a winery in Malaysia. Trypticase soy agar (TSA) (with ambient air incubation) and TSA supplemented with haemin and NADH (with CO_2 enhanced incubation) were used for the isolation of bacteria. The plates were incubated at 37°C for 3 days. A random selection of bacterial isolates were Gram stained and identified using the BD BBL Crystal Identification Systems. *Kytococcus sedentarius* and *Micrococcus luteus* were the predominant bacterial species identified from indoor air. These bacteria were present at relatively high concentrations in indoor air, at times, above 800 colony forming units per cubic meter (CFU/m^3) of air. This indicates that both *K. sedentarius* and *M. luteus* can survive a wide range of adverse conditions, including chemical contamination and ultraviolet exposure. *M. luteus* is a known cause of pneumonia in immunocompromised individuals and has also been implicated in skin infections. Recent reports suggest species of kytococci as emerging opportunistic pathogens of the immunocompromised, paediatrics and the elderly. We postulate that opportunistic bacteria, such as the kytococci and the micrococci, may also have a potential role in instigating subclinical, more subtle symptoms of disease in inmmunocompetent individuals.

INTRODUCTION

Biological contaminants are important determinates of Indoor Air Quality (IAQ). They can be major contributors to health problems such as upper and lower air way diseases and even death as in Legionnaire's disease (Seltzer, 1994). These biological contaminates are potential sources of adverse health consequences even when dead (Burrell, 1991; Radon *et al.*, 2002) and include algae, bacteria, fungi, viruses, protozoa, insects (e.g. cockroaches), arachnids (house dust mite) and pollen (Niven *et al.*, 2002).

Indoor Air Quality (IAQ) profiles differ in various work environments. This is because the source and quantity of the contaminants depends on the existing work procedure (Jensen *et al.*, 2003; Zhang & Smith, 2003). Levels of indoor contaminates may be significantly higher in factory environments as compared to office environments. However, the use of personal protective materials is usually integrated into the work process in factories to limit workers' exposure. Office workers are sometimes faced with various health conditions ranging from minor discomfort to more acute health problems.

Indoor dampness, the presence of stagnant water and high relative humidity provide the necessary source of nutrients and favourable conditions for bacterial growth (Garijoa *et al.*, 2008). Some bacterial contaminants are brought indoors by infected persons. This may result in case-to-case transmission of the infection as in the case of tuberculosis caused by *Mycobacterium tuberculosis* which affects the lungs. *M. tuberculosis* can also be transmitted through the air-management system within a building (Seltzer, 1994; Jones, 1999). Strong winds from the outdoors can also aerosolise microbial contaminants from the soil and other surfaces. These aerosols may infiltrate the indoor environment through various entry points in the building including the ventilation system (Yassin & Almouqatea, 2010).

Microorganisms isolated in winery air have been shown to be related to the particular wine-making process at that time. Apart from the fact that airborne microorganisms in wineries are potential wine contaminants, they could also be hazardous to human health (Picco & Rodolfi, 2004). The grape surface during fermentation usually has a rich micro-flora composed of undesirable and desirable micro-organisms necessary for fermentation. This micro-flora can be aerosolised within the industrial indoor environment, increasing the microbial loads indoors (Zollinger *et al.*, 2006). Bacteria (being a part of the normal microbial flora in wineries) are present throughout the vinification process, either in the wine on the winery equipment (Garijoa *et al.*, 2008).

Although there are many studies on the effects of chemical pollutants in electronic factories, only a few studies have assessed microbial exposure. During the manufacturing process, the fluid used for metal works can form small droplets of mist that are suspended in the air. This mist can be an irritant to the eyes, nose and throat; smaller droplets can penetrate the lungs causing various respiratory problems (Stephen, 2003). Hence, the oil mist generated in electronic or metal working factories might be a potential hazard not only because of its chemical content but also because of its microbial load. There may even be a synergistic effect of both chemical and biological air contaminants. Toxic chemicals may damage the ciliated epithelium of the respiratory tract thereby increasing the penetration of biological allergens. Toxic chemicals may also potentiate the effect of biological agents by suppressing the immune system. Hence, occupational cases of allergic alveolitis caused by biological aeroallergens may be aggravated by chemical factors in the work environment (Hameed *et al.*, 2000; Skorska *et al.*, 2002). It was observed in a study by Hameed (2000) that gaseous chemicals may preserve or kill airborne microorganisms.

This study is an investigation into the type and concentrations of bacteria in the indoor air and outdoor air environments of an office, a winery and an electronic factory and related health effects. It further intended to investigate the relationship between the different indoor activities and the bacterial profile, providing base line data on airborne bacteria in the indoor work environment. This may serve as a reference for future investigations on the role of common airborne bacterial infections in building occupants. Previous studies on bacterial microflora suggest many airborne bacteria are not pathogenic in the immunocompetent host (Yassin 2010). However, some are well known opportunistic pathogens in the immunocompromised host. These opportunistic pathogens might emerge as true pathogens in the near future as environmental changes provide opportunities for the emergence of new pathogens (Cleaveland 2007). Hence, this study aims to establish a base line for further studies on emerging pathogens from indoor air.

MATERIALS AND METHODS

Building profiles

The office is located in a tertiary educational institution in the Federal Territory, Malaysia, whilst the winery and electronic factories are both located in Kajang, Selangor State,

Malaysia. The construction profiles were obtained by site inspection and interview of the maintenance staff and other occupants.

The office had a staff strength of 14. The indoor space was a relatively comfortable environment with adequate ventilation and illumination. It was always clean and there were no unpleasant odours. The winery had a staff strength of 17. Wine was produced through a fermentation process and was subsequently bottled for sale. The winery was cleaned on a routine basis, although it was generally stuffy. The winery had GMP (Good Manufacturing Practice) certification. It complied fully with guidelines on aspects of production that affected product quality. The guidelines included elimination of bioaerosols from air. However, bioaerosol clearing was limited to certain areas; it was not carried out at the fermentation and filtration areas. Both sites operating hours are between 8.30 am to 5.30 pm although most workers stay over-time at the office.

The electronic factory had a staff strength of above 100. It manufactured electronic products such as hard disk parts and other computer components. In addition to the various chemicals and solvents used in the production process, the oil based coolant used generated oil aerosols in the air (oil mist) during the cooling process. The oil mist made the electronic factory and the immediate outdoor environment stuffy. Although most of the electronic factory workers used personal protective equipment, there were complaints of various types of irritation and discomfort. These irritations and discomforts were aggravated by the noise and the heat generated by the manufacturing machines.

Health survey and questionnaire administration

A questionnaire was used to collate data on the health status of workers. The questionnaire design was based on a Singapore IAQ survey questionnaire with relevant modifications appropriate for the study sites. The questionnaire contained questions on demographics, working conditions, discomfort and health complaints. A study information sheet and written consent form with English, Chinese and Malay translations were attached to each questionnaire. Respondents were obligated to fill in the consent form before participating in the study. Since the onset of most symptoms associated with IAQ is within a few days to months of occupation of a building, only workers who had worked at the study site for a minimum of 6 months were included in the study (Allermann *et al.*, 2003). This criterion was set in view of the fact that six months of repeated and cumulative exposure should be adequate for inducing the onset of health symptoms associated with poor IAQ. Workers with chronic health conditions were excluded from the study.

Sampling protocol

Air sample volumes ranging from 100 to 250 litres were drawn using an Ideal Air Sampler (Biomerieux BBL). Six sampling events were conducted over a six month period. The identification of air-borne bacterial species isolated was conducted from two of those six sampling events. There were two sampling points within the office and one sampling point outside the office. Five sampling points were positioned at various locations within the winery, whilst one sampling point was positioned outside. Sampling points 1 to 4 were at the fermentation and filtration areas whilst sampling point 5 was located at the distillation area. There were six sampling points in the electronic factory and two sampling points outside the electronic factory. Point one was located at the packaging area whilst point two was at the loading area. Points three to six were positioned at the manufacturing area. Points seven and eight were the outdoor sampling points. All samples were collected at a height of 1.5m from ground level which is the normal breathing level (Hameed *et al.*, 2000; Oppliger *et al.*, 2005).

Trypticase soy agar (TSA) was used for the isolation and sub-culturing of non-fastidious bacteria (Pastuszka *et al.*, 2000). TSA enhanced with haemin (5ml/L), NADH (5ml/L) and CO_2 (TSA-HN) was used for the isolation and sub-culturing of fastidious

bacteria. Cycloheximide was added to the medium at a concentration of 12 ml/L to inhibit saprophytic fungal growth (Pastuszka *et al.*, 2000). After air samples were collected, the plates were transferred to the laboratory for incubation. TSA plates were incubated at 37ºC for 3 days in ambient air, whilst TSA-HN plates enhanced with haemin (5ml/L) and NADH (5ml/L) were incubated with CO_2 supplementation at 37ºC for 3 days. After incubation, emergent colonies were counted and the total bacterial load in colony forming units per cubic meter of air (CFU/m^3) was determined. Twelve emergent isolates were then randomly selected from each indoor point and 16 emergent isolates were randomly selected from outdoor points and subcultured (Adhikari *et al.*, 2004). A total of 47 isolates, originating from the air inside the office, and 27 isolates, originating from the air outside the office, were identified. One hundred and sixteen (116) isolates, originating from air inside the winery and 32 isolates, originating from air outside the winery, were identified. One hundred and thirty eight (138) isolates were identified from inside the factory whilst forty seven (47) isolates were identified from the outside of the factory. The selected isolates were Gram stained and identified using one or more of the BBL Crystal Identification Systems i.e. the BBL Crystal™ Gram Positive ID System, the BBL Crystal™ Enteric/Nonfermenter ID System and / or the.BBL Crystal™ *Neisseria/ Haemophilus* ID System.

RESULTS

Electronic factory

The concentrations of each bacterial species, expressed in CFU/m^3 of air, are as shown in Figure 1. *Kytococcus sedentarius* (49.2% of the total identified species on TSA-HN indoors) and *Micrococcus luteus* (30.2% of the total identified species on TSA-HN indoors) were the dominant species indoors while *M. luteus* (46.1% of the total identified species on TSA-HN outdoors), was the dominant species outdoors. Gram positive cocci were predominant (97.8% indoors, 87.8% outdoors). Gram positive rods were identified at the low proportions of 1.4% indoors and 10.2% outdoors whilst Gram negative cocci were not isolated in the two sampling events. Gram negative rods were isolated at 0.7% and 2.0% indoors and outdoors respectively. Common symptoms of workers in the electronic factory included cough (63.1%), sneezing (61.5%), fatigue (53.8%), headache (50.8%) and back/neck pain (61.5%).

Office

The results for bacterial identification are shown in Figure 2. The commonly identified bacteria in indoor air were *M. luteus* (64.3% of the total identified species on TSA-HN indoors), *K. sedentarius* (23.0% of the total identified species on TSA-HN indoors) and *Staphylococcus schleiferi* (16.2% of the total identified species on TSA indoors) whilst *M. luteus* (81.8% of the total identified species on TSA-HN outdoors) and *Leuconostoc lactis* (19.7% of the total identified species on TSA outdoors) were the most abundant bacteria detected in outdoor air. The Gram staining results show that Gram positive cocci were predominant in both indoor as well as outdoor air (comprising 93.6% of the bacteria found in indoor air and 92.6% of the bacteria found in outdoor air). The Gram negative cocci (2.1% indoors, 0.0% outdoors), Gram positive rods (2.1% indoors, 7.4% outdoors) and Gram negative rods (2.1% indoors, 0.0% outdoors) were isolated in very low proportions. The most prevalent health symptoms of the office workers were sneezing (70.6%) and back/ neck pain (64.7%) whilst no occupant reported nasal bleeding.

Winery

The concentrations of bacteria identified in the winery are shown in Figure 3. Identified bacteria from indoor air included *M. luteus* (67.4% of the total identified species on TSA-HN indoors) and *K. sedentarius* (12.3% of the total identified species on TSA-HN indoors). *K. sedentarius* (34.3% of the total identified species on TSA-HN outdoors) was the dominant species outdoors. In line with the observations at the office, Gram positive

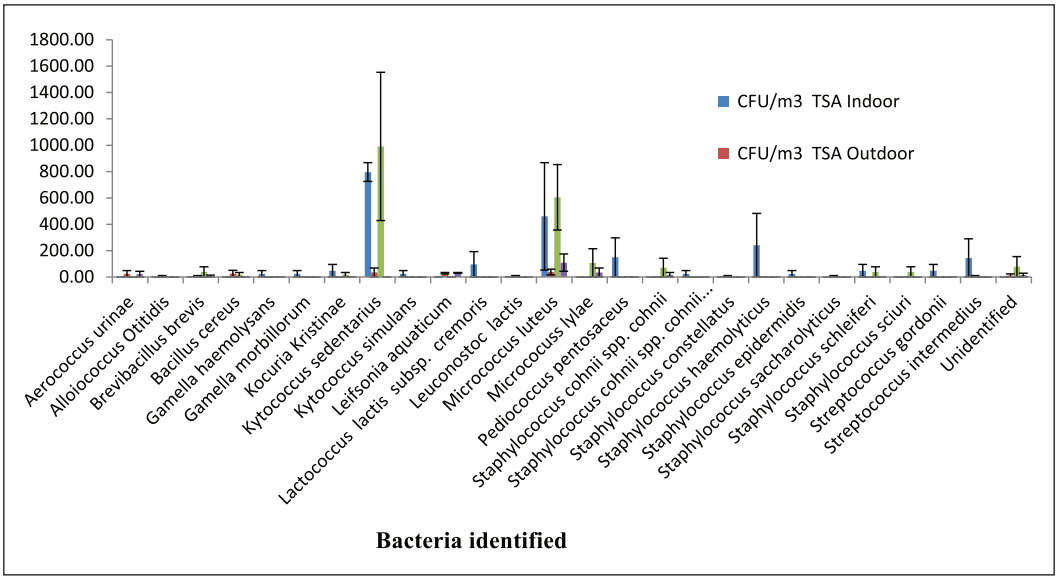

Figure 1. Average bacterial load observed on TSA and TSA-HN in the first and second samples collected at the indoor and outdoor sampling points of the electronic factory expressed in CF.

Abbreviations and symbol used on chart
TSA: Trypticase soy agar.
TSA-HN: Trypticase soy agar with CO_2 enhanced incubation.
CFU/m^3: Colony forming units per cubic meter of air.
I: Standard deviation bar.

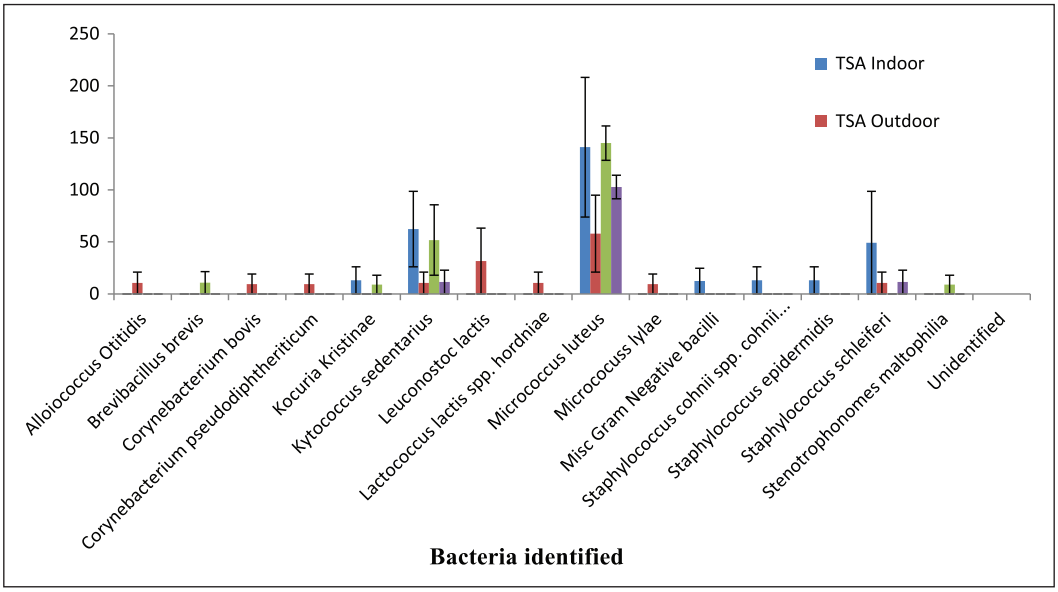

Figure 2. Average bacterial load observed on TSA and TSA-HN in the first and second samples collected at the indoor and outdoor sampling points of the office expressed in CFU/m^3.

Abbreviations and symbol used on chart
TSA: Trypticase soy agar.
TSA-HN: Trypticase soy agar with CO_2 enhanced incubation.
CFU/m^3: Colony forming units per cubic meter of air.
I: Standard deviation bar.

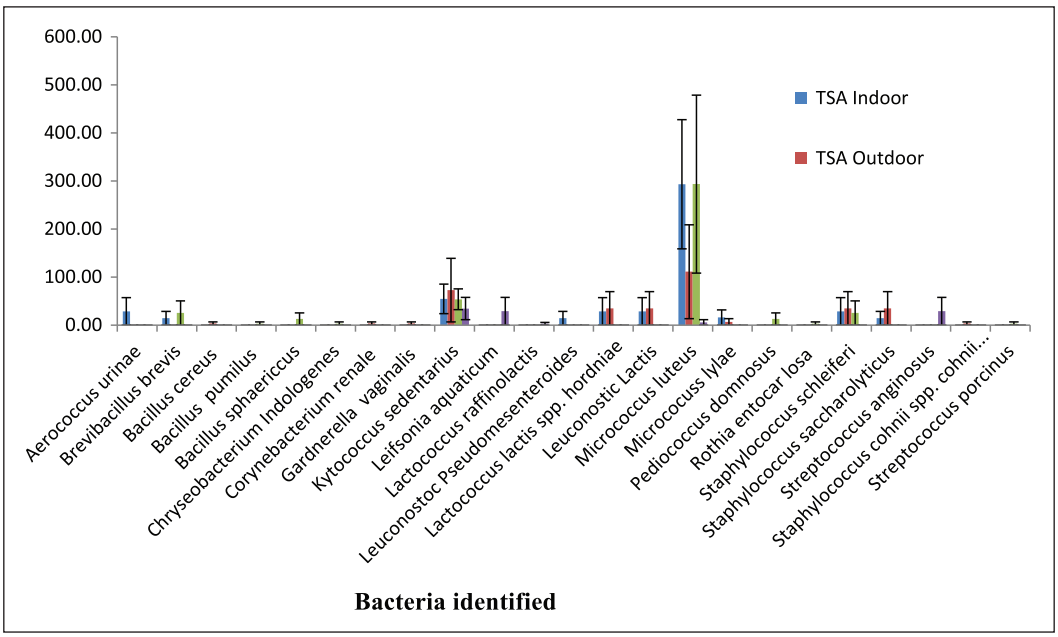

Figure 3. Average bacterial load observed on TSA and TSA-HN in the first and second samples collected at the indoor and outdoor sampling points of the winery expressed in CFU/m^3.

Abbreviations and symbol used on chart
TSA: Trypticase soy agar.
TSA-HN: Trypticase soy agar with CO_2 enhanced incubation.
CFU/m^3: Colony forming units per cubic meter of air.
⌶: Standard deviation bar.

cocci were predominant in the winery (92.2% indoors, 87.5% outdoors). Gram positive rods were identified at lower proportions (5.2% indoors, 9.4% outdoors) whilst Gram negative cocci and Gram negative rods were seldom isolated. Frequent thirst (64.2%) and back/neck pain (64.2%) were the prevalent health symptoms amongst winery workers. There were no reports of sinous congestion, wheezing, skin rashes, ear irritation, diarrhoea, fatigue or difficulty in remembering things.

DISCUSSION

Gram positive rods were the second most abundant type of bacteria at the winery after Gram positive cocci. *B. brevis* (14.3 CFU/m^3 on TSA, 25.2 CFU/m^3 on TSA-HN), *Rothia dentocariosa* (3.2 CFU/m^3 on TSA-HN) and *Bacillus pumillus* (3.2 CFU/m^3 on TSA-HN) are airborne Gram positive rods that were found inside the winery. *R. dentocariosa* is generally benign in nature, but can, upon occasion, cause infectious disease. Other than dental caries, from which it was first isolated, *R. dentocariosa* can cause endocarditis (Ricaurte *et al.*, 2001), endopthalmitis (MacKinnon *et al.*, 2001), infections of the brain and intercranial tissues (Ricaurte *et al.*, 2001), tonsils (Ohashi *et al.*, 2005), cornea (Morley *et al.*, 2006), peritoneum (Morris *et al.*, 2004) and lungs (Ricaurte *et al.*, 2001). *Aerococcus urinae*, a Gram positive coccus, was not isolated from office air but it was isolated from the winery air at a low concentration (28.5 CFU/m^3 of air). It is a rare pathogen known to cause urinary tract infection. Gram negative rods found inside the winery included *C. indologenes* (3.2 CFU/m^3 on TSA-HN) and *Bacillus sphaericus* (12.6 CFU/m^3 on TSA-HN). *C. indologenes* has been implicated in

bacteraemia in patients with indwelling medical devices (Hsueh *et al.*, 1996) whilst *B. sphaericus* is primarily an insect pathogen. *Gardnerella vaginalis* (associated with bacterial vaginosis), *Bacillus cereus* and *Corynebacterium renale* (a known animal pathogen) are Gram positive rods that were present outdoors of the winery. *Bacillus* and *Corynebacterium* are genera associated with cutaneous infections (Aydogdu 2005).

The variety in species identities in the winery is likely to be related to the wine manufacturing processes. This is because the wine-making process involves interactions between various microorganisms resulting in a series of biological reactions. Bacterial loads were generally higher at the first sampling event, especially for *M. luteus*. The first sampling event happens to coincide with the peak of production in the winery. Garijoa *et al.* (2008) observed that bacterial load is related to the rate of microbial activities in a winery.

The Gram positive coccus, *M. luteus*, was the dominant bacterium in the office (141.03 CFU/m^3 on TSA, 145.0 CFU/m^3 on TSA-HN) and in the winery (293.0 CFU/m^3 on TSA, 293.4 CFU/m^3 on TSA-HN). It is known to be a nosocomial pathogen implicated in pulmonary arterial hypertension, meningitis, skin infection (in HIV positive patients) and pneumonia (Oudiz, 2004). The Gram positive coccus, *K. sedentarius*, an opportunistic pathogen, was the second most dominant bacterium found at the two study sites (Office; 62.3 CFU/m^3 on TSA, 51.8 CFU/m^3 on TSA-HN; Winery; 141.0 CFU/m^3 on TSA, 145.0 CFU/m^3 on TSA-HN). Its adverse health effects are described in relation to findings in the electronic factory discussed below. *K. sedentarius* was formerly classified as *Micrococcus sedentarius* and shares similar physiological profiles with *M. luteus*. *Micrococcus* spp. was the dominant species in indoor air in a study by Fang in Beijing (Fang *et al.*, 2007), which is similar to observations made in this study. *K. sedentarius* was the second dominant species outside the office (10.5 CFU/m^3 on TSA, 11.4 CFU/m^3 on TSA-HN) and winery (72.9 CFU/m^3 on TSA, 34.3 CFU/m^3 on TSA-HN) as well. Clearly, the abundance of this species is not limited to the indoor environment. It tends to endure in adverse outdoor conditions as well. The bacterial loads of *K. sedentarius* were constantly higher indoors than outdoors in most observations. This may be largely influenced by the bactericidal effect of ultraviolet light outdoors (Burrel 1991, Selter 1994).

The significance of the abundance of *M. luteus* in the winery (293.0 CFU/m^3 on TSA, 293.4 CFU/m^3 on TSA-HN) and office (141.0 CFU/m^3 on TSA, 145.0 CFU/m^3 on TSA-HN) cannot be undermined. In addition to its dominance, the load of *M. luteus* was very high-reaching 478.0 CFU/m^3 of air in the winery as observed on TSA-HN plates at the first sampling event. Given its high load, *M. luteus* may be partly, although perhaps not exclusively, implicated as a cause of some of the identified health symptoms amongst workers that were observed in this study. Fifty nine percent (58.8%) of the respondents in the office had dry skin. *M. luteus*, a normal part of the skin microflora, has also been implicated in skin infections. It may be playing a role in manifesting symptomatic dry skin amongst the workers in the office. Pathogenicity studies are required to affirm this relationship.

The synergistic effects of other environmental conditions must also be considered. Toxic chemicals may damage the epithelium of the skin thereby facilitating the penetration of biological agents or potentiating the effect of biological agents by suppressing the immune system. *M. luteus* could be a potential skin pathogen even in immunocompetent building occupants when acting synergistically with other contaminates.

M. luteus is known to cause pneumonia in immunocompromised individuals. However, its role in causing respiratory health symptoms should also be investigated in immunocompetent building occupants. This is particularly important in view of the high prevalence of respiratory tract symptoms in the office such as stuffy nose (58.8%), runny nose (58.8%), sneezing (70.6%) and cough (52.9%). The role of *K. sedentarius* in

causing the prevailing health symptoms detected amongst workers is similar to that of *M. luteus* discussed earlier. Both bacteria have been implicated in skin infections and pneumonia.

Gram positive cocci were dominant inside and outside the electronic factory. Gram positive rods and Gram negative cocci occurred only in small proportions. Gram negative cocci were not isolated both in the indoors and the outdoors. They tend to be fragile and intolerant to adverse environmental conditions. However the fact that no Gram negative cocci were isolated does not suggest their absence. This data is in accordance with the observation by Zollinger *et al.* (2006). Some bacteria, such as *Staphylococcus epidermidis*, *S. hemolyticus*, *S. schleiferi*, *S. sciuri*, *Streptococcus gordonii*, *Gemella haemolysans*, *G. morbillorum*, *Kocuria kristinae*, *Kytococcus simulans* and *Lactococcus lactis* subsp. *cremoris*, were only isolated from indoor air. They were not found in outdoor air, whilst others like *Aerococcus urinae*, *Alloiococcus otitis*, *Leifsonia aquatica*, *Leuconostoc lactis*, *Streptococcus constellatus* and *Staphylococcus saccharolyticus* were only present in outdoor air. The presence of wide and open exit/entry points clearly underpinned the easy influx and efflux of bacteria in and out of the electronic factory. However, the presence of a particular bacterium is suggestive of a preference for the habitat that it is found in. The bacteria in indoor air may tolerate and perhaps even favour indoor conditions such as the presence of chemicals and other solvents. The same set of bacterial species may not tolerate outdoor air conditions, such as the bactericidal effect of ultraviolet light. Most of the bacteria found outdoors were isolated only at low concentrations. The light intensity outdoors was higher than indoors reducing the chances of survival of some bacterial species in the outdoors. However, contrary to the latter observations, counts of the Gram positive cocci, *K. sedentarius* and *M. luteus* were markedly different. *K. sedentarius* and *M. luteus* were prevalent both in indoor and outdoor air, suggesting a positive adaptation against ultraviolet radiation. This makes the survival abilities of these Gram positive cocci a subject of interest. In addition to the relative predominance of these bacteria in the indoors in comparison to other bacterial species, the indoor bacterial loads of both *K. sedentarius* and *M. luteus* were distinctly high i.e. at times, exceeding 800.0 CFU/m^3 of air. This indicates that both *K. sedentarius* and *M. luteus* can survive a wide range of adverse conditions, including chemical contamination indoors as well as ultraviolet exposure outdoors. It must be borne in mind that microbial counts exceeding 500 CFU/m^3 in an indoor air-conditioned environment is deemed 'unclean' according to guidelines by the Institute of Environmental Epidemiology, Singapore, as well as the Hong Kong Indoor Air Quality Objective (HKIAQO) Level 1 standard.

K. sedentarius has been reported to produce the oligoketide antibiotics monensins A and B (Pospisil 1998). The antibacterial properties of these monensins may have contributed to the survival and predominance of *K. sedentarius* in the air by antagonistic action against other microbial genera. In addition to its remarkable survival abilities, *K. sedentarius* may also become pathogenic under certain conditions. *K. sedentarius* has been implicated in skin infections caused by extracellular enzymes that degrade human callus, a condition known as pitted keratolysis (Longshaw *et al.*, 2002). Recent reports suggest species of kytococci as emerging opportunistic pathogens of the immunocompromised, paediatrics and the elderly. To our knowledge, eleven human infections due to members of the genus *Kytococcus* or *Kytococcus*-associated infections have been reported thus far. Levenga *et al.* (2004) report *K. sedentarius* associated fatal haemorrhagic pneumonia in a 55 year old neutropenic man suffering from acute myeloid leukaemia. Greene *et al.* (1980) report cerebral cyst infection and ventriculoatrial shunt infection with *M. sedentarius* (currently known as *K. sedentarius*) in a 7 year old boy with congenital hydrocephalus.

In gauging the relative pathogenicity of the kytococci, it is important to note that they are generally resistant to penicillin G, methicillin and isoaxazoyl penicillins (Levenga *et al.*, 2004). Levenga *et al.* (2004) report that the clinically isolated *K. sedentarius* causing fatal haemorrhagic pneumonia was resistant to penicillin, cefuroxime, cefepime, methicillin, gentamicin and clarithromycin. The route of kytococcal infections is not, as yet, clear and optimal empirical therapy has not been established.

These reports cumulatively suggest that pure cultures of kytococci from paediatric patients, the immunocompromised or neutropenic patients and the elderly, should not be viewed as merely contaminants, but rather, may be the underlying cause of serious and life-threatening infections. Therefore, should there be guidelines as to acceptable limits of the kytococci and other opportunistic bacteria in indoor air? If they are colonisers of the human skin and mucosa, they will be shed into indoor environments on a continual basis. In closed air-conditioned environments, the possibility of bacterial bioaccumulation is a reality. Indoor reservoirs of some of these opportunistic bacteria, for example of the kytococci, in rodents, insects, carpets and upholstery have yet to be thoroughly examined. If present at high concentrations indoors, persons leaving such indoor environments will have a net increase in bacterial colonisation. If they are within the high risk group for contracting kytococcal or other opportunistic infections, this will render them vulnerable to infection. At the very least, they may become carriers of infection to those in the high risk category. As such, we propose that acceptable limits for the kytococci in indoor air should be studied and established. On the same note, acceptable limits for other established opportunistic pathogens commonly found in indoor air should also be similarly determined.

Fifty nine percent (58.8%) of the respondents in the office had dry skin. This is possibly due to the synergistic effects of some indoor contaminants. Toxic chemicals may damage the epithelium of the skin thereby facilitating the penetration of biological agents or potentiating the effect of biological agents by suppressing the immune system. *M. luteus* could be a potential skin pathogen in building occupants when acting synergistically with other contaminates. *M. luteus* is also known to cause pneumonia in immunocompromised individuals. This may be related to the high prevalence of respiratory tract symptoms in the office such as stuffy nose (58.8%). Hence, its role in respiratory health symptoms should also be investigated. The health impact of inhalable bacteria is quite significant because a large variety of bacterial species exist indoors (Jones, 1999). As a whole, the underlying cause(s) of the health symptoms observed in the workers at all three study sites may not be monofactorial but rather multifactorial in origin.

CONCLUSIONS

M. luteus and *K. sedentarius* were by far the most predominant bacteria detected in this study. Their abundance in the air both indoors and outdoors at all three sites of study signifies their tolerance of a diverse array of environmental conditions. They have been clearly implicated in skin infections and pneumonia in immunocompromised individuals. The possibility exists that interactions between microorganisms present in indoor air and chemicals released in the workplace have contributed to some of the health symptoms observed such as dry skin and respiratory tract symptoms, even in immunocompetent workers. Although there is an increasing pool of data available on the indoor airborne microbial flora, their definitive association with the health status of occupants has not been fully established. More comprehensive studies are necessary, especially in the work environment, where people are exposed to indoor air for long periods and are at high risk of developing adverse effects. This study has provided important base line data on airborne bacteria in three indoor work environments i.e. an

office, a winery and an electronic factory, in tropical, equatorial Malaysia. This may serve as a reference for future investigations on the role of common airborne bacteria in infections in immunocompetent and immunocompromised building occupants. In gauging the potential adverse health significance of high loads of opportunistic pathogens in the indoor air of work environments, we must bear in mind that there is ample evidence of opportunistic pathogens causing debilitating disease and mortality in high risk groups such as the immunocompromised. However, their potential role in instigating subclinical, more subtle, symptoms of disease (eg. chronic fatigue syndrome) in apparently healthy individuals, has yet to be established and would be an interesting area of study given their predominance in the indoor air of all three sites of study. Mukamalova *et al.* (1998) report that *M. luteus* secretes a bacterial cytokine/pheromone (resuscitation promoting factor, *rpf*) that can resuscitate both dormant cells of its own as well as that of *Mycobacterium tuberculosis*, *M. avium*, *M. bovis*, *M. kansasii*, *M. smegmatis* and certain other Gram-positive bacteria. Thus, we will end this paper with the suggestion that this *rpf* secreted by *M. luteus* colonised and infected individuals *MAY* be enhancing growth and infectivity of other potential pathogens in the human body, leading to the symptoms of sick building syndrome, chronic fatigue syndrome and other idiopathic syndromes associated with modern day indoor living.

Abbreviations

CFU/m^3: colony forming units per cubic meter; HKIAQO: Hong Kong Indoor Air Quality Objective; IAQ: Indoor Air Quality; TSA: Trypticase soy agar; TSA-HN: TSA enhanced with haemin (5ml/L), NADH (5ml/L) and with CO_2 augmented incubation.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

A. Folayan undertook all experimentation and drafted the manuscript, K. Mohandas and S. Ambu revised the manuscript, Verasingam Kumarasamy, Nagaraja Lee and J.W. Mak were members of the student supervisory team. All authors read and approved the final manuscript.

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